UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/725,309	11/29/2000	Alok Singh	79,212	8594

7590 12/03/2002

Associate Counsel (Patents), Code 1008.2
Naval Research Laboratory
Washington, DC 20375-5000

EXAMINER
HUTSON, RICHARD G

ART UNIT	PAPER NUMBER
1652	

DATE MAILED: 12/03/2002

9

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/725,309	SINGH ET AL.	
	Examiner	Art Unit	
	Richard G Hutson	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *18 September 2002* .

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-21* is/are pending in the application.

4a) Of the above claim(s) *1 and 2* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *3-21* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Applicants amendment of the specification, and claims 3, 5, 9, 11 and 12, and the addition of new claims 15-21, Paper No. 8, 9/18/2002, is acknowledged.

Claims 1-21are still at issue and are present for examination.

Applicants' arguments filed on 9/18/2002, Paper No. 8, have been fully considered and are deemed to be persuasive to overcome some of the rejections previously applied. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

Claims 1 and 2 remain withdrawn from further consideration by the examiner, 37 CFR 1.142(b), as being drawn to a non-elected invention, the requirement having been traversed in Paper No. 6.

This application contains claim1 and 2 drawn to an invention nonelected with traverse in Paper No. 6. A complete reply to the final rejection must include cancellation of nonelected claims or other appropriate action (37 CFR 1.144) See MPEP § 821.01.

Specification

The disclosure is objected to because of the following informalities:

The amendment filed 9/18/2002 is objected to under 35 U.S.C. 132 because it introduces new matter into the disclosure. 35 U.S.C. 132 states that no amendment shall introduce new matter into the disclosure of the invention. The added material which is not supported by the original disclosure is as follows: Applicants amendment which recites "A 'stabilizing amino acid substitution' is a substitution that non-covalently

bonds to IDA salts or NTA salts without substantially effecting the catalytic function of the enzyme." This statement is not supported by the original disclosure.

Applicant is required to cancel the new matter in the reply to this Office Action.

Applicants amendment to the specification beginning at page 5, line 19 to include recite "As used herein, the term 'amino acid substitution' includes both the addition of one or more amino acid residues to a protein without removing any residues as well as changing one or more residues in a protein to other residues." is confusing as discussed below under 112 second paragraph rejection. It is believed that applicants "definition is repugnant to the usual meaning of the term substitution. That is a substitution is considered to be a replacement of one amino acid with another. The adding of amino acid residues is standardly termed insertion or addition.

Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 3-14 and 15-21 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The rejection is stated in the previous office action, with respect to claims 3 (4-8 dependent on) and 9 (10-14 dependent on) and the recitation "...genetically engineering an enzyme to include a stabilizing amino acid substitution...".

Applicants traverse this rejection on the basis that the specification has been amended in the paragraph beginning at page 5, line 19 to include the definition of "amino acid substitution"

> "As used herein, the term 'amino acid substitution' includes both the addition of one or more amino acid residues to a protein without removing any residues as well as changing one or more residues in a protein to other residues."

Applicants submit that this definition includes both the addition of one or more amino acid residues to a protein without removing any residues as well as changing one or more residues in a protein to other residues. Applicants argument is not found persuasive on the following basis.

While applicant may be his or her own lexicographer, a term in a claim may not be given a meaning repugnant to the usual meaning of that term. See *In re Hill*, 161 F.2d 367, 73 USPQ 482 (CCPA 1947). The term "substitution" in the claims is used by the claim to mean "substitution and or addition" while the accepted meaning is "substitution."

Applicants state that the above definition explicitly states what is implicit in the previous sentence, "The enzymes are genetically engineered to include a poly-His tail as well as other stabilizing amino acid substitutions." Applicants submit that "the poly-His tail" is an addition and then conclude that this is one form of substitution. This argument is not found persuasive. A substitution is the replacement of one or more amino acid residues with an equal number of different residues and an addition is

considered to be the inclusion of amino acid residues not previously present without any loss of the residues originally present.

Claims 17 and 20 are indefinite in the recitation "...known to be innocuous to the function of the enzyme..." as this recitation is unclear based on these claims being drawn to the methods of claims 3 and 9, respectively. This confusion is in light of claims 16 and 19 which are also drawn to the same methods of claims 3 and 9, respectively , and the same as claims 17 and 20 in every respect, with the exception that claims 16 and 19, rather then limit the methods as the above recitation, limit the methods "...innocuous to the function of the enzyme...". Thus the only difference between claim 16 and 17 and claims 19 and 20 is that the binding site is "known" in claims 17 and 20, whereas in claims 16 and 19, while the site must be "innocuous", it might not be "known to be innocuous". What is "known" changes with time and cannot be clearly defined. Furthermore, what is "known" to one person, may not be "known " to others.

Claims 16, 17, 19 and 20 are indefinite in that the recitation "at a binding site on the enzyme" is unclear. It is not understood what type of "binding site" applicants are referring to. It is confusing if the recited "binding site" is meant to be encompass a "substrate" binding site, a "cofactor" binding site or the site on the enzyme which the enzyme itself is bound to the salt.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 3-14, and 15-21 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The rejection was stated in the previous office action as it applied to claims 3-14.

Applicants traverse this rejection on the basis that at page 6, lines 8-10, the application states that "enzymes that can be used for this technique are those enzymes that have appropriately reactive surface available histidines or which have a histidine tag that can be added through site specific mutagenesis. This includes polyhistidine." Applicants further point out that the protein can be genetically engineered to have histidine on its surface, including at the termini or at internal residues and the substitutions are not limited to histidine and polyhistidine. Applicants argument is not found persuasive because while applicants appear to argue that they have disclosed that a specific portion of the protein (i.e. the surface) should be focused on, in making the changes of the claimed invention, applicants have not described any specific changes encompassed within this genus, with the exception of the addition of histidine residues at the amino terminus of the *E. coli* enzyme thioesterase I. Such a specific mutation (i.e. the addition of histidine residues on the amino terminus of the *E. coli* enzyme thioesterase I), is in no way representative of the infinitesimally large number of methods of genetically engineering, encompassed by applicants claim.

Thus, applicants have failed to sufficiently describe the claimed invention, in such full, clear, concise, and exact terms that a skilled artisan would recognize applicants were in possession of the claimed invention.

Applicant is referred to the revised guidelines concerning compliance with the written description requirement of U.S.C. 112, first paragraph, published in the Official Gazette and also available at www.uspto.gov.

Claims 3-14 and 15-20 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method for stabilizing an enzyme comprising genetically engineering an enzyme to include a polyhistidine sequence on the amino or carboxyl terminus, does not reasonably provide enablement for any method for stabilizing an enzyme comprising genetically engineering an enzyme to include any stabilizing amino acid substitution. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The rejection was stated in the previous office action.

Applicants traverse this rejection on the basis that the application does provide guidance as to selection of a site on a protein for genetically engineering a substitution. Applicants submit that the main criterion is that the site of the substitution (the binding site) on the enzyme be far away from or innocuous to the function of the enzymes catalytic site. Further in support of applicants position applicants submit that they disclose bovine carbonic anahydrase, which naturally contains histidine residues, and

thus provides guidance on how to genetically engineer substitutions on a protein. Applicants further argue that the art is well knowledgeable with respect to the prediction of the structure of a protein by comparing the sequence of the protein to known sequences with known structures and through the use of computer modeling and applicants support this position with the references, Zhang and Lu et al.

Applicants argument is not found persuasive because as was stated previously, while recombinant and mutagenesis techniques are known, as well as methods of predicting protein structure based on amino acid sequence alone, it is not routine in the art to screen for multiple substitutions or multiple modifications, as encompassed by the instant claims, and the positions within a protein's sequence where amino acid modifications can be made with a reasonable expectation of success in obtaining the desired activity/utility are limited in any protein and the result of such modifications is unpredictable. Applicants merely teach the addition of a polyhistidine sequence to the amino or carboxyl termini of a "genetically engineered protein" or the use of histidine residues located "naturally" on the surface of the enzyme. In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, e.g. multiple substitutions.

The specification does not support the broad scope of the claims which encompass all methods for genetically engineering any stabilizing amino acid substitution into an enzyme structure because the specification does not establish: (A) those enzymes or regions of those enzymes' structure which may be modified effecting stability without effecting activity; (B) the general tolerance of said enzyme to

modification and extent of such tolerance; (C) a rational and predictable scheme for modifying any amino acid residue of said enzyme with an expectation of obtaining the desired biological function; and (D) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful. While applicants have asserted that they have provided guidance to determine which substitutions would be acceptable to effect the enzyme stabilization claimed, as the relationship between the sequence of a peptide and its tertiary structure (i.e. its activity and stabilization) are not well understood and are not predictable (e.g., see Ngo et al. in The Protein Folding Problem and Tertiary Structure Prediction, 1994, Merz et al. (ed.), Birkhauser, Boston, MA, pp. 433 and 492-495, Ref: U, Form-892), it would require undue experimentation for one skilled in the art to arrive at the majority of those methods of the claimed genus having the claimed means of enzyme stabilization.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 9-12, 14 and 19-21 are rejected under 35 U.S.C. 102(b) as being anticipated by Qiagen Product Guide, 1997, pages 106-110.

The rejection is stated in the previous office action as applied to claims 9-12 and 14.

Newly added claims 19-21 are drawn to the method of stabilizing enzymes of claim 9, wherein the method is further limited in that the stabilizing amino acid substitution is at a binding site on the enzyme that is innocuous or known to be innocuous to the function of the enzyme (claims 19 and 20) and wherein the stabilizing amino acid substitution is terminal polyhistidine (claim 21).

With respect to these additional added limitations, Qiagen Product Guide teaches the QIAexpress Ni-NTA (nickel-nitrilotriacetic acid) protein purification system and methods for using this system comprising genetically engineering the insertion of a cDNA sequence encoding a desired protein (to be purified) sequence into an expression vector pQE, such that it inserts a 6X histidine(polyhistidine) tag into the protein. As taught by Qiagen the 6Xhis tag rarely affects protein structure or function, and need not be removed from the purified protein, thus these claims are included in this rejection.

Applicants traverse this rejection on the basis that the purpose of the process taught by Qiagen is to purify the tagged protein and Qiagen does not disclose whether the tagged protein can perform any catalytic function while attached to the Ni-NTA base material. Applicants further point out that the definition of "stabilizing amino acid substitution" as used in claim 9 states that the enzyme non-covalently binds to IDA salts or NTA salts without substantially affecting the catalytic function of the enzyme. Applicants assert that Qiagen differs from the invention of claim 9 in that Qiagen does not disclose that the attached protein has any catalytic activity. This argument is not found persuasive. Applicants argue that "the definition of stabilizing amino acid

substitution as used in claim 9 states that the enzyme non-covalently binds to IDA salts or NTA salts without substantially affecting the catalytic function of the enzyme." It is noted that this definition, which was added to the specification in the previous response, and discussed above under 112 second paragraph rejection, states "A 'stabilizing amino acid substitution' is a substitution that non-covalently bonds to IDA salts or NTA salts without substantially effecting the catalytic function of the enzyme." (See page 2, Paper No. 8, 9/18/2002). This definition makes no reference that the "bound" enzyme must be catalytically active, only that the substitution non-covalently bonds to IDA salts or NTA salts and the substitution does not substantially effecting the catalytic function of the enzyme. Thus while Qiagen does not specifically disclose that the attached protein has catalytic activity "while bound", such a characteristic is not a limitation of the rejected method claims. It is not a necessary limitation that each of these "characteristics" be performed simultaneously, hence such a limitation in the claim, as argued by applicants, is not persuasive.

Further, Qiagen does teach that the 6Xhis tag rarely affects protein structure or function and need not be removed from the purified protein. Thus the inclusion of a limitation that the bound enzyme must retain catalytic activity while bound, would likely result in an obviousness type rejection, as the ordinary artisan is well skilled in the use of the above Qiagen method of protein immobilization/stabilization.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claim 13 is rejected under 35 U.S.C. 103(a) as being unpatentable over Qiagen Product Guide, 1997, pages 106-110, as applied to claims 9-12 and 14 above, and further in view of Lu et al. (Journal of Biological Chemistry, Vol 271, No. 9, pages 5059-5065, March 1996).

The rejection is stated in the previous office action.

Applicants traverse this rejection as above on the basis that neither Qiagen nor Lu et al. discloses that the enzyme binds to IDA salts or NTA salts without substantially affecting the catalytic function of the enzyme. Applicants argument is not persuasive for the same reasons discussed above under the 102 rejection over Qiagen, that such a characteristic is not a limitation of the rejected method claims.

Claims 3-5, 7, 8 and 15-18 are rejected under 35 U.S.C. 103(a) as being unpatentable over Singh (U.S. Patent Number. 5,663,387), LeJeune et al. (Biotechnology and Bioengineering Vol 54, No. 2, pages 105-114, April 1997) and Polayes et al. (Life Technologies-FOCUS, Vol 16, page 81-84. July 1994).

The rejection is stated in the previous office action as it applied to claims 3-5, 7 and 8.

Newly added claims 15-18 are drawn to the method of stabilizing enzymes of claim 3, wherein the method is further limited in that the bound enzyme is capable of detoxifying a nerve agent (claim 15), wherein the stabilizing amino acid substitution is at a binding site on the enzyme that is innocuous or known to be innocuous to the function of the enzyme (claims 16 and 17) and wherein the stabilizing amino acid substitution is terminal stabilizing amino acid substitution (claim 18).

With respect to these additional added limitations, as was previously stated, one of ordinary skill in the art at the time of filing would have been motivated to immobilize a nerve agent hydrolyzing enzyme, such as phosphotriesterase, as a method of enhancing the stability of the enzyme as taught by LeJeune et al. LeJune et al. further teach that phosphotriesterase, a nerve-agent hydrolyzing enzyme, is well documented in the literature, and LeJune suggests that any means used to increase the stability of the enzyme should involve sites on the enzyme that are innocuous to the function of the enzyme. Polayes et al. further teach that no change in enzymatic activity (CAT activity) was observed upon removal of a terminal polyhistidine tag, thus indicating that the terminal tag did not interfere with enzymatic function.

In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208

USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

Applicants traverse this rejection on the following basis. Applicants submit that Singh does not disclose genetically engineering the enzyme by any method including the addition of a poly-His tail. This is acknowledged, Polayes et al. is relied upon for this teaching. Polayes et al. teach methods of genetically engineering the incorporation of a polyhistidine sequence at either the amino or carboxyl terminus of a protein. Applicants further assert that Singh also does not disclose the use of a lipid having a NTA group. This is acknowledged, Polayes et al. is relied upon for this, and further Singh teaches a lipid having a IDA group and applicant is reminded that the claim 3 is drawn to a method which includes the use of either a IDA **or** NTA group and the use of both of these different groups supports that they are functionally equivalent.

Applicants argue that the motivation as previously stated is not found in the references and that the references teach away from this combination.

Applicants state that Singh teaches away from using any method of covalently binding the enzyme and that that LeJune only disclose covalent methods of binding the protein. This is acknowledged . Applicants therefore assert that a person skilled in the art who was aware of Singh would not look to LeJune to modify Singh. However, applicant is reminded that LeJune is only relied upon to teach that "protein immobilization is a common method of enhancing enzyme stability" and that the specific enzyme, phosphotriesterase, a nerve-agent hydrolyzing enzyme, is an example of such an enzyme stabilized by immobilization. Thus LeJune is not used to modify Singh, but

to show motivation to immobilize other enzymes which lack characteristics needed for the use of Singh's methods. Those characteristics (i.e. the presence of histidine amino acid residues) being inherent to Singh's method. Applicant is reminded that Singh also teaches that the immobilization of molecular assemblies and proteins, using polymerizable phospholipids is a means of stabilization.

Applicants further assert that Singh also lacks a suggestion to genetically engineer the enzyme to add a poly-His tail to the enzyme. This is acknowledged, as stated in the previous office action, Polayes et al. is relied upon for this teaching. Polayes et al. teach methods of genetically engineering the incorporation of a polyhistidine sequence at either the amino or carboxyl terminus of a protein. Singh merely used carbonic anahydrase as an example of a protein which may be bound to the taught liposomes. As an example of an enzyme which could be bound to the outer surface of the taught vesicles, Singh teach the use of an enzyme which contains several exposed histidine residues, for example, carbonic anhydrase II, since it contains six histidine residues, four of them available within a distance of 6A. There is no need to add a polyhistidine tail to an enzyme which has a sufficient number of exposed histidine residues available for binding. However, not all enzymes have the necessary number of exposed histidines, hence the motivation to add a polyhistidine tail as taught by Polayes et al., to those enzymes which are in need of histidine residues for binding.

Finally applicants submit that Polayes lacks a suggestion to use the methods of either Singh or LeJune, as in the Qiagen and Lu references, Polayes is only concerned with purifying a protein. As previously stated, Polayes et al. is only relied upon for its

teaching of methods of genetically engineering the incorporation of a polyhistidine sequence at either the amino or carboxyl terminus of a protein and that this 6 histidine sequence has a strong affinity for the Ni^{2+}-nitrilo-triacetic acid resin.

Conclusion

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

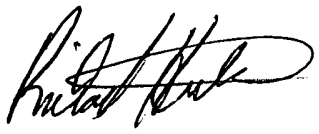
A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Richard G Hutson whose telephone number is (703) 308-0066. The examiner can normally be reached on 7:30 am to 4:00 pm, M-F.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapu Achutamurthy can be reached on (703) 308-3804. The fax phone numbers for the organization where this application or proceeding is assigned

are (703) 305-3014 for regular communications and (703) 305-3014 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.



Richard Hutson, Ph.D.
Patent Examiner
Art Unit 1652
December 2, 2002